

Appendix B:

Synthetic Control Design Parameters: Exemplary Ligation Extension Components and Reaction Parameters

1. Components (20 μ l reaction)

| | |
|---|-------------|
| CF ligation mixture | 10 μ l |
| Bridge template (PCR amplicon / concentrated bridge oligo) | 5.0 μ l |
| Reference nucleic acid mix (0.5 μ M) | 5.0 μ l |

2. Reaction Parameters

| | | |
|-------|------------|-----------|
| 90° | 5 seconds | 32 Cycles |
| 46.5° | 45 seconds | |
| 99° | 10 minutes | |
| 4° | ∞ | |
| | | |